Kidney International, Vol. 41 (1992), pp. 1262–1273

Modulation of glomerular proteoglycans by insulin-like growth factor-1

YUZO WATANABE, NAOKI KASHIHARA HIROFUMI MAKINO, and YASHPAL S. KANWAR

Department of Pathology, Northwestern University Medical School, Chicago, Illinois

Modulation of glomerular proteoglycans by insulin-like growth factor-1. Effect of insulin-like growth factor-1 (IGF) on the synthesis of glomerular proteoglycans (PGs) in an ex vivo recirculating organ perfusion system was investigated. Kidneys were perfused with a medium (~80 ml) containing [^{35}S]-sulfate (250 μCi/ml) and IGF (62.5 to 625 ng/ml). After radiolabeling, a small cortical piece was saved for tissue autoradiography, and the remaining kidney and the perfusion medium were utilized for biochemical studies. The glomeruli were isolated; their PGs extracted and characterized. A two- to threefold increase of the total radioactivities in tissue and media fractions was observed with the exposure to IGF. By Sepharose CL-6B chromatography, the tissue PGs eluted as two peaks (A and B) with Kav = 0.24 and 0.48, and the majority of the radioactivity was confined to peak A. This peak contained intact PGs while peak B included glycosaminoglycan (GAG) chains. Elution profiles of the glomerular PGs were similar in the control and IGF groups. However, there was a disproportionate increase of chondroitin/dermatan sulfate in the IGF group. The media fractions also had two peaks, and most of the radioactivity was associated with peak B containing GAG chains. A remarkable accentuation of peak B along with significant increase in the chondroitin/dermatan sulfate were observed in the IGF group. By DEAE-Sephacel chromatography, the PGs/GAGs of IGF group eluted at a relatively lower salt concentration as compared to the control. Autoradiography revealed a relatively high concentration of radioactivity over the mesangium as compared to the other cell types of the glomerulus. [^{35}S]-methionine studies revealed a generalized increase of protein synthesis in the IGF group, but comparatively much less than that of PGs/GAGs. These results indicate that IGF enhances the biosynthesis of PGs/GAGs by various cell types of the renal glomerulus, especially that of the mesangial cell, as reflected by the selective increase of chondroitin/dermatan sulfate.

Insulin-like growth factor-1 (IGF-1) is a single polypeptide chain (Mr ~7,600) whose protein sequence and diverse biological activities are similar to those of insulin [1–6]. Its biological effects are mediated via specific plasmalemmal receptors present on a wide variety of cells, including those of renal glomeruli and tubules [7–9]. The presence of these receptors on renal mesangial cells has led several investigators to believe that IGF-1 may play a role in renal hypertrophy in diabetes and after partial renal ablation, perhaps by modulating the metabolism of various glycoproteins of the extracellular matrix (ECM) [10–13].

Received for publication May 17, 1991
and in revised form November 25, 1991
Accepted for publication December 3, 1991

The glomerular ECM, comprised of mesangial matrix (MM) and glomerular basement membrane (GBM), consists of several glycoproteins: type IV collagen, laminin, entactin and proteoglycans [14–17]. Several studies have delineated the derangements in the metabolism of one or more glycoproteins of the ECM in diabetic nephropathy [18, 19]. The latter is accompanied by the expansion of the mesangium and thickening of the GBM. The biochemical abnormalities related to these renal morphological lesions seem to reside in the collagenous and proteoglycan components of the ECM [20, 21]. In this regard, a decrease in the biosynthesis of proteoglycans has been reported in both experimental and clinical diabetic states [21–24]. Incidentally, these changes in the ECM are presumed to lead to a proteinuric response observed in diabetic nephropathy [25]. Along with the changes in the ECM composition, the IGF-1 concentrations have been shown to increase in certain target tissues, that is, retina and kidney [12, 26]. Thus, it is conceivable that the IGF-1 or insulin modulates the metabolism of some of the glycoproteins of the ECM which leads to defective cell-matrix interactions and ultimately to the evolution of characteristic morphologic lesions seen in diabetic nephropathy [27].

Little is known about the influence of IGF-1 on the metabolism of ECM components, particularly of the proteoglycans. So far the information available in the literature pertains to cultured chondrocytes [28, 29]. The influence of IGF-1 on the metabolism of renal proteoglycans is unknown. In this investigation, we studied the effect of IGF-1 on the biosynthesis of proteoglycans by various cell types of the glomerulus.

Methods

Radiolabelling of the proteoglycans

An ex vivo organ perfusion system was utilized for radiolabeling of renal proteoglycans [15, 30, 31]. Rationale for using this system was to "mimic" the in vivo conditions while excluding the effects of various growth factors or hormones, circulating in the blood, on the kidney. This system also overcame the inherent difficulties in the interpretation of the biosynthetic data obtained from various cell or glomerular culture experiments where a change in the phenotypic expressions was observed [32].

The right kidney of 250 g male Sprague-Dawley rats was isolated from the systemic circulation. The renal artery was cannulated, blood was flushed out of the kidney with oxygenated KRB-buffer containing 7.5% BSA, and the kidney was

Table 1. Total incorporated [^{35}S]-sulfate radioactivity in tissue and media fractions

IGF concentration *ng/ml*	Glomerular[a] (*N* = 5)	Media[b] (*N* = 5)
0	7.83 ± 0.58	6.49 ± 0.73
62.5	9.16 ± 0.47[c]	9.84 ± 0.78[c]
125	10.31 ± 0.35[c]	12.12 ± 0.90[d]
375	12.94 ± 0.68[d]	15.73 ± 0.95[d]
625	16.02 ± 0.79[d]	18.29 ± 1.02[d]

Data are presented as means ± SD.
[a] The counts are given as 10^7 DPM in glomeruli isolated from one kidney
[b] The counts represent 10^5 DPM per ml of the perfusion medium
[c] $P < 0.01$
[d] $P < 0.001$

connected to a recirculating organ perfusion system. The perfusion medium (~80 ml) consisted of modified Krebs-Ringer bicarbonate (KRB) solution containing a mixture of amino acids, 10 mM D-glucose and 7.5% bovine serum albumin. The perfusion was carried out for nine hours under well-oxygenated and isothermic conditions. [^{35}S]-sulfate (NEN Products, Boston, Massachusetts, USA) with a specific activity >1000 Ci/mmol was included in the perfusion medium at a concentration of 250 μCi/ml to label the proteoglycans. The final inorganic sulfate and glucose concentrations were adjusted to 50 μM and 7.5 mM, respectively. Varying concentrations of human recombinant IGF-1 (62.5 to 625 ng/ml; Amgen Biologicals, Thousand Oaks, California, USA) was added to the perfusion medium to ascertain its effect on the biosynthetic profiles of the proteoglycans. At the end of the nine-hour labelling period, the kidneys were perfused with KRB-buffer containing 7.5% BSA for 15 minutes. A small (1 × 4 mm) cortical biopsy was performed and processed for tissue light and electron microscopic autoradiography. The kidneys were then flushed with Hank's balanced salt solution containing 1% BSA, a mixture of protease inhibitors (5 mM benzamidine-HCl, 1 mM phenylmethylsulfonyl fluoride, 100 mM 6-aminohexanoic acid and 10 mM N-ethylmaleimide) and 50 mM EDTA. Following that, they were bisected, their cortices dissected out and frozen at −20°C. The perfusion medium was also saved after the addition of protease inhibitors for isolation and characterization of proteoglycans (PGs) and glycosaminoglycans (GAGs).

Isolation of glomeruli and extraction of proteoglycans

Frozen cortices were thawed and glomeruli isolated by the sieving technique in the presence of protease inhibitors at 4°C. The glomerular PGs were extracted with 10 volumes of 4 M GuCl solution containing 10 mM sodium acetate, 50 mM EDTA, 0.1% CHAPS and a mixture of protease inhibitors, pH 5.8 [15, 31]. Extraction was carried out by rigorous continuous stirring for 48 hours at 4°C. The unextracted fraction was collected as a sediment by centrifugation at 10,000 rpm for 15 minutes. The sediment was hydrolyzed with 0.5 N NaOH at 45°C for three hours to extract the remaining incorporated radioactivity in the glycosaminoglycans. Aliquots of both the extracts were applied to Sephadex G-25 (Pharmacia Inc., Piscataway, New Jersey, USA) column equilibrated with the elution buffer [4 M GuCl, 0.1 M Tris-HCl, 0.1 M Na_2SO_4, 0.5% (vol/vol) Triton X-100, pH 7.0), and the total incorporated radioactivity in the excluded fractions was determined. Finally, the mean radioactivity and standard deviation about the mean were determined of the data obtained from five separate experiments per variable.

The remaining extracts were dialyzed successively against 0.15 M NaCl containing a mixture of protease inhibitors and ice-cold distilled water. Subsequently, aliquots were prepared, lyophilized and stored at −20°C.

Characterization of extracted glomerular proteoglycans

The characterization of PGs was carried out by Sepharose CL-6B and DEAE-Sephacel chromatographies (Pharmacia, Inc.) as detailed in our previous publications [15, 31]. Some of the lyophilized aliquots were either digested with chondroitinase-ABC (Miles Laboratories, Naperville, Illinois, USA) or with purified heparitinase (Sigma Chemical Co., St. Louis, Missouri, USA) as previously described [33]. The lyophilized aliquots were reconstituted with 150 μl of 4 M GuCl and applied to Sepharose CL-6B columns (100 × 0.9 cm), equilibrated with the same elution buffer used for Sephadex G-25 chromatography. Vo (void) and Vt (total) volumes of Sepharose CL-6B column were determined by employing [^{3}H]-serine cartilage monomer proteoglycans and [^{3}H]-serine, respectively. After application of the reconstituted extracts to the column, the radioactivity in the eluted fractions was measured, following which the chromatographic profiles of the untreated and enzymatically-treated PGs were prepared. The molecular weights of the glomerular PGs were estimated by interpolation of the data obtained from tryptic-chymotryptic digests of cartilage proteoglycans [34].

DEAE-Sephacel columns were prepared in disposable serological pipettes with a bed volume of 2 ml, and equilibrated with elution buffer containing 8 M urea, 0.1 M NaCl, 0.05 M sodium acetate and 0.1% CHAPS, pH 6.0. The lyophilized samples were reconstituted with the elution buffer and applied to the DEAE-Sephacel column. Elution was carried out with the same buffer containing a continuous gradient of 0.1 M to 1.0 M NaCl, and effluent fractions were collected and radioactivity determined.

Isolation and characterization of glomerular glycosaminoglycans

Glycosaminoglycans (GAGs) were prepared from the lyophilized tissue proteoglycans by alkaline hydrolysis with 0.5 N NaOH at 45°C for three hours. The hydrolysates were dialyzed against ice-cold distilled water, aliquots prepared and lyophilized. The characterization was carried out by Sepharose CL-6B chromatography before and after treatment with heparitinase or chondroitinase-ABC as previously described [33]. The molecular weights of the GAG chains were estimated by comparing the peak elution positions with the values obtained by Wasteson for chondroitin sulfate [35].

Isolation and characterization of media proteoglycans/glycosaminoglycans

Aliquots of 0.5 ml were applied to Sephadex G-25 column, the excluded fractions collected, pooled and total incorporated radioactivity in the media determined. The media PGs/GAGs from the excluded fractions were characterized by Sepharose

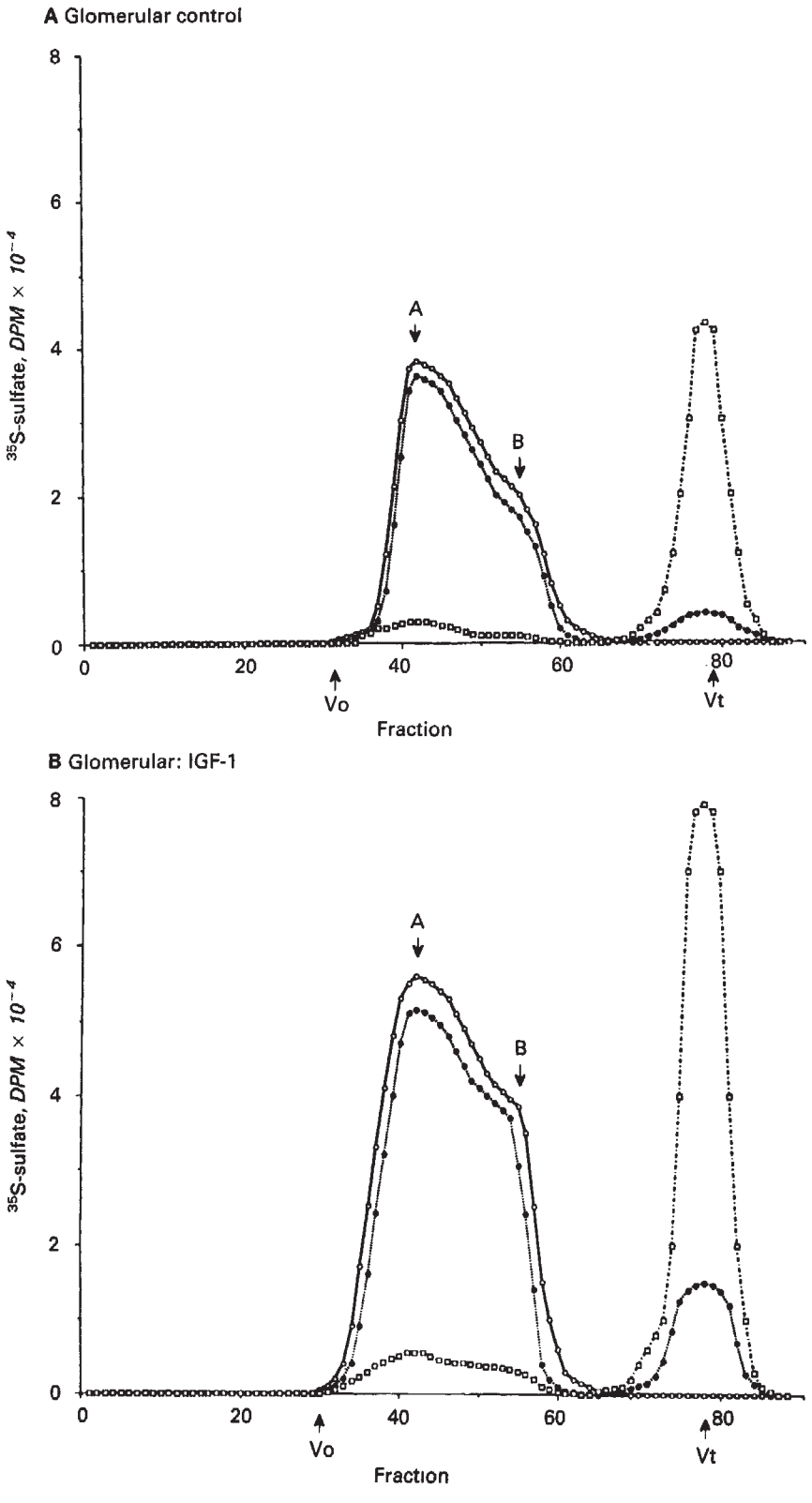

Fig. 1. *Sepharose CL-6B chromatograms of [^{35}S]sulfate-labelled proteoglycans/glycosaminoglycans extracted from glomeruli of control and IGF-treated kidneys.* In control (panel A), the intact (original, —○—) PGs eluted as 2 peaks (A and B) of radioactivity. Treatment with heparitinase (--□--) and chondroitinase-ABC (C-ABC, —●—) resulted in the release of ~93% and ~7% of the radioactivities into the Vt fraction, respectively. In the IGF-group, the elution profiles of PGs/GAGs were similar to control but a greater amount of radioactivity (~26%) was released into the Vt fraction with the treatment of chondroitinase-ABC.

CL-6B and DEAE-Sephacel chromatographies before and after digestion with chondroitinase-ABC or heparitinase as described above.

Experiments with [^{35}S]-methionine

These studies were carried out to ascertain the effect of IGF-1 on generalized protein synthesis. The rat kidneys were radio-

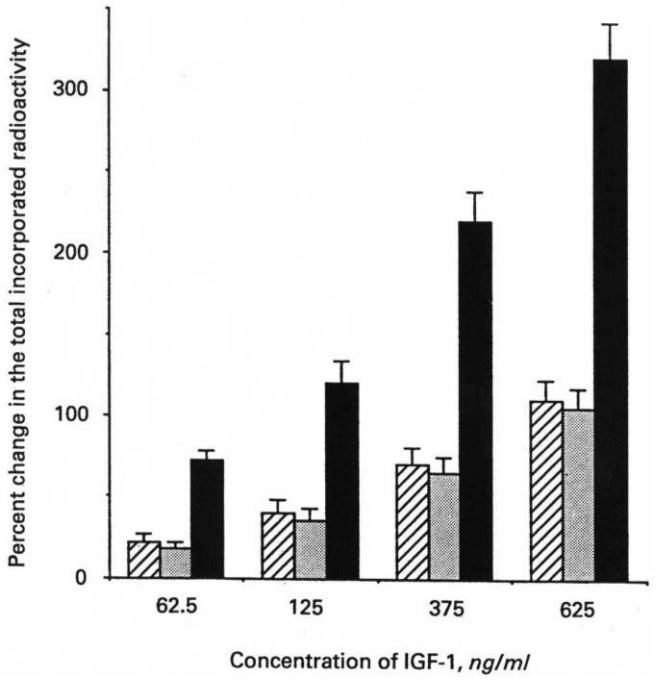

Fig. 2. *Effect of various concentrations of IGF-1 on the synthesis of [^{35}S]-labelled total PGs (glomerular + media; ▨), HS-PG (▧) and CS/DS-PG (■).* Maximal effect was observed in CS/DS-PG fraction at all concentrations of IGF used. $N = 5$.

labelled with inclusion of [^{35}S]-methionine (10 μCi/ml) and IGF-1 (375 ng/ml) in a modified medium of the organ perfusion system as described above. The modification included exclusion of the methionine from the medium. After nine hours of radiolabeling, a small cortical biopsy was performed and the remaining kidneys were processed for isolation of glomeruli, and extraction of proteins and proteoglycans as detailed above. Free [^{35}S]-methionine was removed by passing the extracts through Sephadex G-25 column and total incorporated radioactivity determined. Finally, mean and standard deviation were determined of the data obtained from five experiments.

Tissue autoradiography and morphometric analyses

The cortical biopsy tissues were minced into 1 mm^3 pieces, immersed in an aldehyde fixative, post-fixed in osmium tetroxide, dehydrated in graded series of ethanol and propylene oxide and embedded in EPON [36]. Thick (0.5 μm) as well as thin (60 nm) sections were prepared and processed for respective light and electron microscopic autoradiographies as detailed in our previous publications [30, 36]. The morphometric analyses were carried out on EM autoradiograms only. Thirty-five micrographs from each group were taken and printed to a final magnification of × 5,000. Each micrograph included capillary loops, mesangium and all the three cell types of the glomerulus. Accordingly, four compartments were defined, that is, epithelium, endothelium, mesangium and the glomerular basement membrane (GBM). The mesangium included both the extracellular matrix as well as the mesangial cell. Separate compartments for the mesangial cell and its matrix were not assigned due to the inherent difficulties in accurately delineating the two compartments since the matrix is interspersed among the cytoplasmic processes of the cell. The relative area of each compartment was computed by the point counting method [37], utilizing a transparent overlay of a network of points spaced 1.0 cm apart. The number of intersections over a given compartment represents its relative area. The area occupied by the nucleus was excluded in these determinations. Then, total number of grains were counted on each compartment by the best-fit circle method [38]. Finally, the grain density (concentration of radiation) was calculated by dividing the grain count by total number of intersections. The data from control and IGF-1 groups of various autoradiographic and biochemical studies were statistically analyzed by Student's *t*-test.

Results

Incorporation of [^{35}S]-sulfate into tissue and media fractions

With the efficiency of extraction >96%, the total incorporated radioactivities associated with proteoglycans/glycosaminoglycans (PGs/GAGs) were $7.83 \pm 0.58 \times 10^7$ dpm/kidney and $6.49 \pm 0.73 \times 10^5$ dpm/ml in the tissue and media fractions, respectively (Table 1). With the IGF-1 treatment the incorporated radioactivities increased in both the fractions in a dose dependent manner. However, the increased synthesis in the media fraction appeared to be relatively more as compared with the tissue fraction. Such increases were observed at all concentrations (62.5 to 625 ng/ml) of IGF-1.

Characterization of glomerular proteoglycans and glycosaminoglycans

All chromatographic procedures were carried out in triplicate and representative elution profiles of extracted proteoglycans/glycosaminoglycans (PGs/GAGs) are shown in Figure 1. In the control, a major peak of radioactivity (peak A) with Kav = 0.24 (Mr = 150,000 to 200,000, intact PGs) and a smaller shoulder peak (peak B) with Kav = 0.48 (Mr 20 to 30,000, GAG chains) were observed. Respective treatments of PGs/GAGs with heparitinase and chondroitinase-ABC released ~93% and ~7% of the radioactivities into the Vt fractions (Fig. 1A), indicating thereby that the predominant *de novo* synthesized PGs/GAGs were made up of heparan sulfate-proteoglycan (HS-PG). The amount of chondroitin/dermatan sulfate proteoglycans (CS/DS-PG) in peaks A and B were in proportion to the total incorporated radioactivity included in individual peaks.

The PGs/GAGs from kidneys exposed to IGF-1 showed elution profiles similar to the control but containing relatively more incorporated radioactivities (Fig. 1B). The results of the treatment with heparitinase and chondroitinase-ABC revealed an increase in the *de novo* synthesis of HS-PG as well as that of CS/DS-PG. Although there was an increase in the total incorporated radioactivity associated with proteoglycans, the amount associated with CS/DS-PG was disproportionately higher (Fig. 2). The relatively higher increase in the synthesis of CS/DS-PG was also seen at all concentrations of IGF-1 used (Fig. 2). A significant increase of three to fourfold in the synthesis of CS/DS-PG was noted at a concentration of 625 ng/ml. Finally, the increase in the CS/DS-PG associated radioactivity was observed in both the peaks eluted from Sepharose CL-6B column, that is, peaks A and B. By DEAE-Sephacel chromatography, no differences in the elution profiles were observed between the control and IGF-1-treated groups.

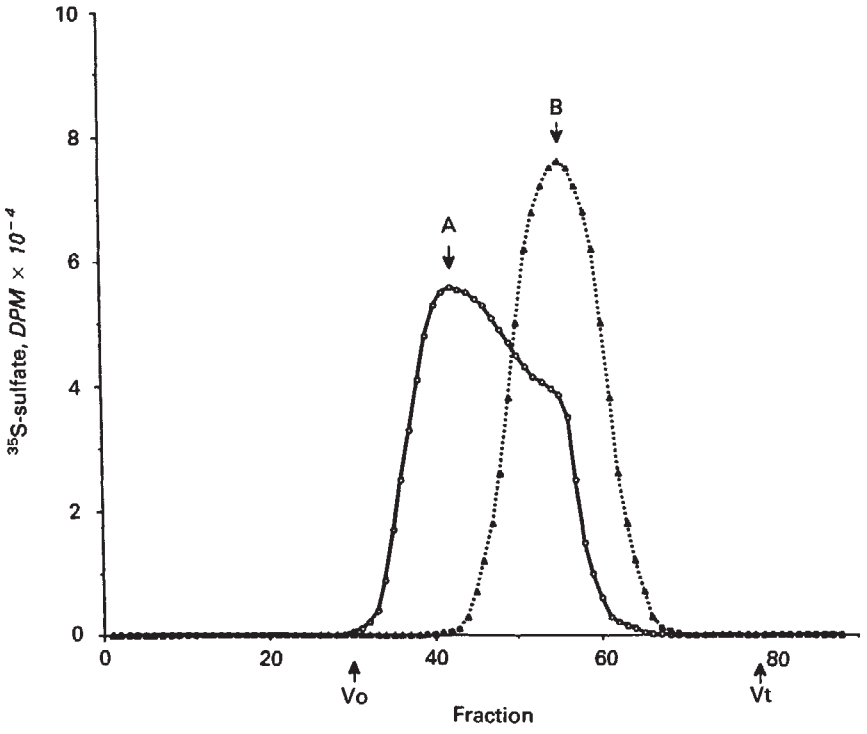

Fig. 3. *Sepharose CL-6B chromatograms of glomerular PGs/GAGs before (original; —○—) and after alkaline hydrolysis (NaOH-Tx; --△--).* Peak A represents intact PGs while peak B contains the GAG chains.

Alkaline treatment of the extracted PGs/GAGs resulted in the release of GAG chains from the intact PGs and appeared as a single peak of radioactivity and Kav shifted from 0.24 to 0.48 (Fig. 3). In these GAG chains, the proportion of HS and CS/DS were similar to those noted in the intact PGs. However, a higher proportion of CS/DS was observed in the IGF group (figure not included).

Characterization of media proteoglycans/glycosaminoglycans

In the control, the elution profiles of media PGs/GAGs (Fig. 4A) revealed two peaks of radioactivity with Kav = 0.24 (peak A) and 0.48 (peak B) by Sepharose CL-6B chromatography. Most of the radioactivity was associated with peak B which contained the GAG chains. Treatment with heparitinase and chondroitinase-ABC released ~90% and ~10% of the radioactivities into the Vt fractions, respectively. The radioactivities released into the Vt fractions were proportionate to the total radioactivities included in peaks A and B. The PGs/GAGs from IGF-1 exposed kidneys had elution profiles similar to that of the control, but contained higher amounts of incorporated radioactivities (Fig. 4B). The accentuation of peak B suggested an increase in the synthesis of GAG chains or accelerated degradation of *de novo* synthesized intact proteoglycans. Furthermore, the percentage of CS/DS-PG in the media fraction increased in a dose dependent manner with increasing concentrations of IGF-1 and were comparable to values in tissue fractions. DEAE-Sephacel chromatography revealed certain differences between the control and experimental groups. The PGs/GAGs of the control group eluted between 0.4 and 0.6 M NaCl, whereas those of the IGF-1-treated group eluted between 0.35 and 0.55 M NaCl of the salt gradient with a left shoulder peak (Fig. 5).

Morphologic and autoradiographic studies

General morphologic features of glomerular epithelium, endothelium, mesangium and of the extracellular matrices were well preserved in both the groups. No discernible morphological alterations were observed in the intracellular organelles of the kidneys exposed to IGF-1. Also, the various cell types of the glomerulus remained adherent to the extracellular matrices.

Light microscopic autoradiograms of the kidneys perfused with [^{35}S]-sulfate revealed an increase in silver grains associated with cellular as well as extracellular components of the glomerulus (Fig. 6 A vs. B). The increase in the autoradiographic grains was more obvious in the mesangial regions. Also, the number of autoradiographic grains appeared to increase more in the mesangial regions with exposure to higher concentration of IGF-1 (Fig. 6 B vs. C vs. D). These differences were more apparent by electron microscopic autoradiography. A remarkable increase in the autoradiographic grains overlying the GBM and mesangium was observed (Fig. 7 A vs. B). Some increase was also observed over the epithelial and endothelial compartments. The autoradiographic grains were mostly localized over the Golgi-saccules of various cell types of the glomerulus—the site where sulfation of the proteoglycans occurs. Morphometric analyses of electron microscopic autoradiograms revealed an almost threefold higher grain density in the mesangial compartment of the kidneys exposed to IGF-1 as compared to the control (Table 2). The increase in the grain density over the GBM and endothelial compartments was relatively less. The epithelial compartment exhibited an increase of 25 to 30% in the grain density (Table 2).

Experiments with [^{35}S]-methionine

Light microscopic autoradiograms revealed a mild to moderate generalized increase in the silver grains overlying the

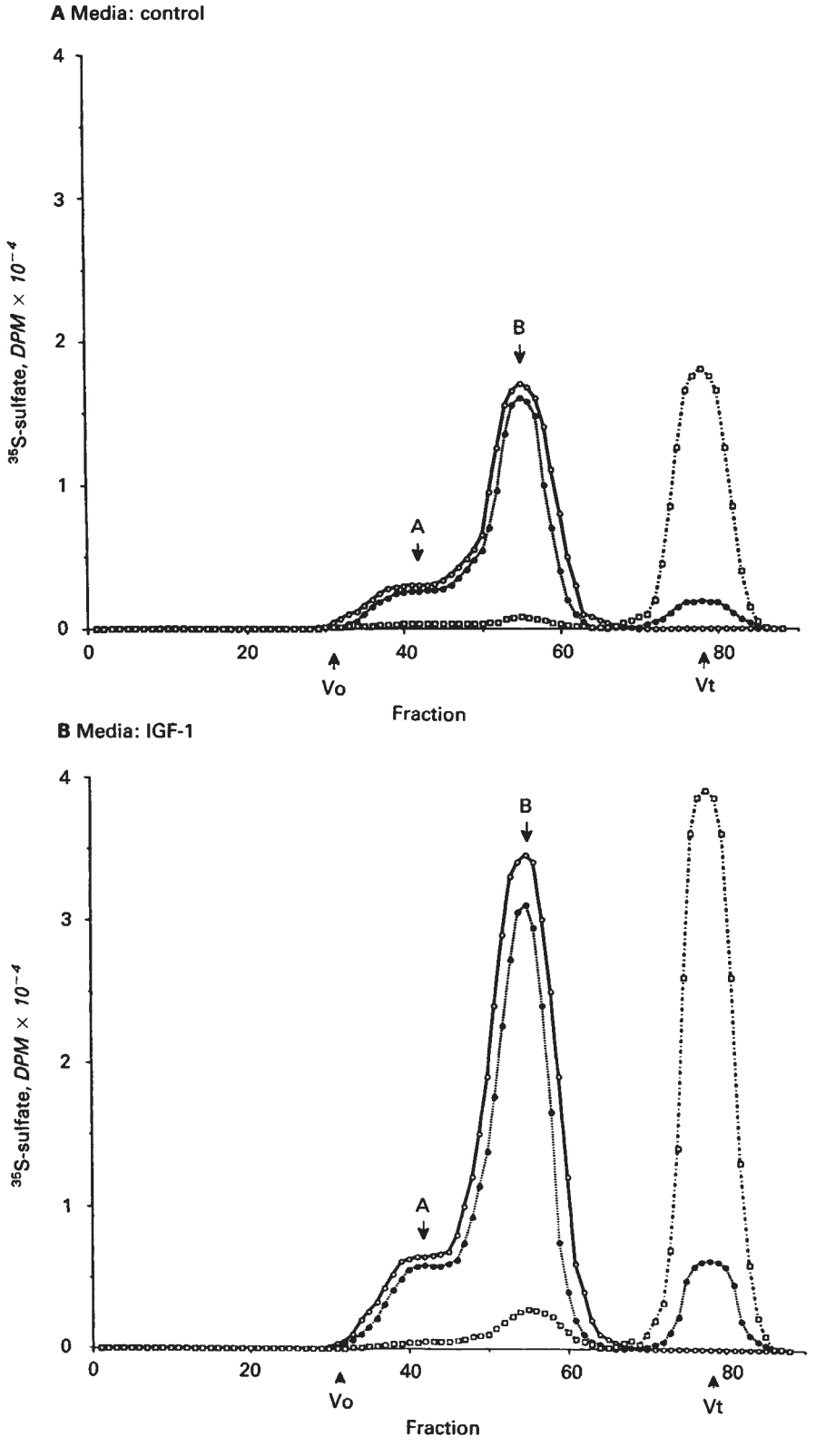

Fig. 4. *Sepharose CL-6B chromatograms of media PGs/GAGs from control (A) and IGF-treated kidneys (B), before (original; —○—) and after treatments with heparitinase (---□---) and chondroitinase-ABC (C-ABC; —●—).*

various compartments of the glomeruli from kidneys exposed to IGF-1 (Fig. 8 A vs. B). By electron microscopic autoradiography, no appreciable differences were observed in the grains overlying the epithelial, endothelial and the GBM compartments (Fig. 8 C vs. D). However, a notable increase was observed in the mesangial compartment of the kidneys exposed to IGF-1 as compared to the control. The grain density analyses confirmed a selective increase in the incorporation of [^{35}S]-

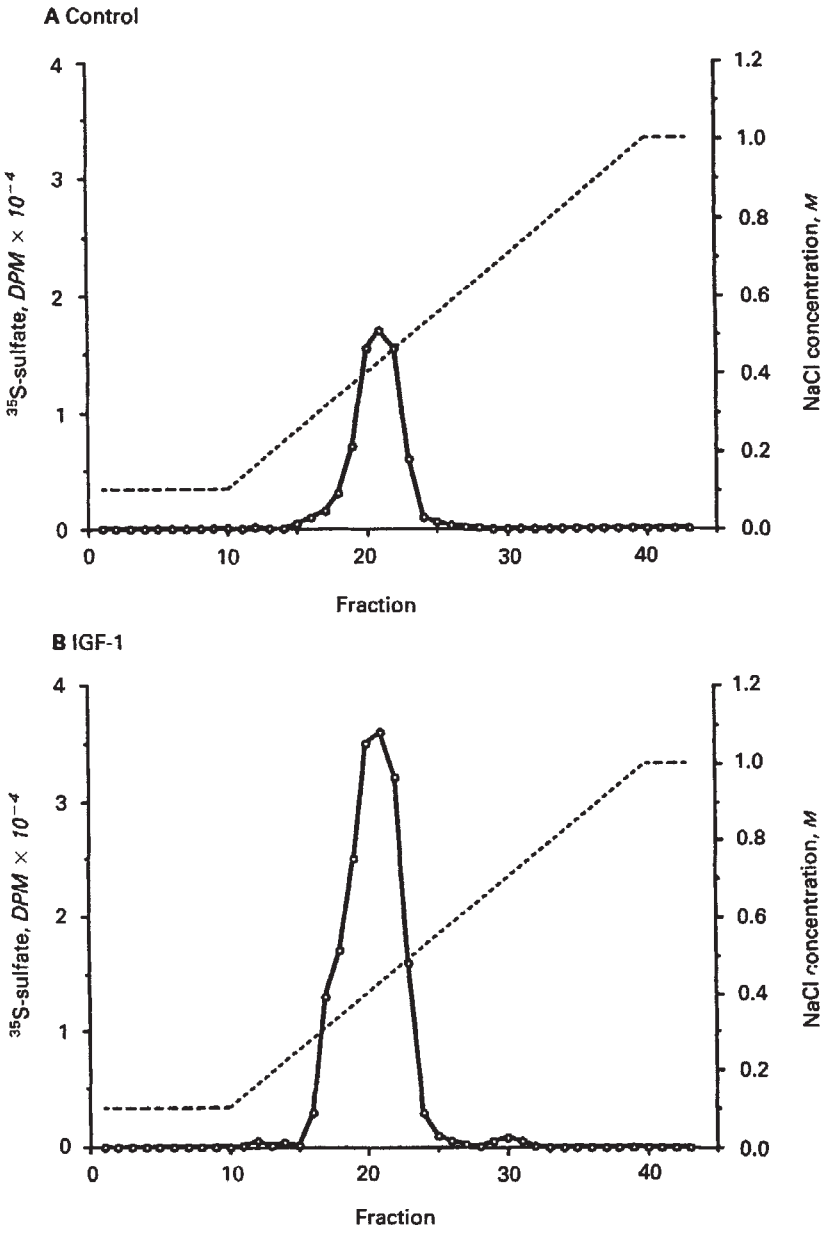

Fig. 5. *DEAE-Sephacel chromatograms of media PGs/GAGs from control (A) and IGF-1-treated (B) groups.* The media PGs/GAGs of IGF-1 group elute at a relatively lower salt concentration as compared to the control (0.35–0.55 vs. 0.4–0.6 M NaCl). Symbols are: (—○—) radioactivity; (---) NaCl.

methionine associated radioactivity over the mesangial compartment of the glomeruli of the IGF-1 group (Table 2). Nevertheless, the grain density of the mesangial compartment associated with [^{35}S]-methionine was much less compared to that of [^{35}S]-sulfate. The biochemical studies further substantiated the data of autoradiographic studies. A slight increase in the incorporation of [^{35}S]-methionine into both the tissue and media fractions was observed in IGF-1-treated kidneys (Table 3). However, the increase in [^{35}S]-methionine incorporation was noticeably less compared to [^{35}S]-sulfate with the exposure to comparable amounts of IGF (Table 4).

Discussion

The results of this investigation indicate that IGF-1 stimulates a generalized *de novo* synthesis of proteins, more so of the proteoglycans, and especially that of chondroitin/dermatan sulfate. The increased synthesis of the latter may be attributable to the stimulatory effect of IGF-1 on the mesangial cells of the renal glomerulus. Although the stimulation was seen in all the cell types of the glomerulus, the effect of IGF-1 seems to be predominantly targeted towards the mesangial cell.

The mesangial cell is located centrally in the renal glomerulus and synthesizes a wide variety of glycoproteins, inflammatory mediators and hormones, and exhibits autocrine and paracrine functions [39, 40]. One of the glycoproteins which is exclusively synthesized by the mesangial cell includes chondroitin sulfate-proteoglycan (CS-PG) [30, 41]. Incidentally, certain species of CS-PGs synthesized by cultured mesangial cells have similarities to decorin and biglycans [42, 43]. In any event, the increased synthesis of CS/DS-PG observed here may be reflective of the enhanced biosynthetic activity of the mesangial cell under the influence of IGF-1. Also, following IGF-1 stimulation

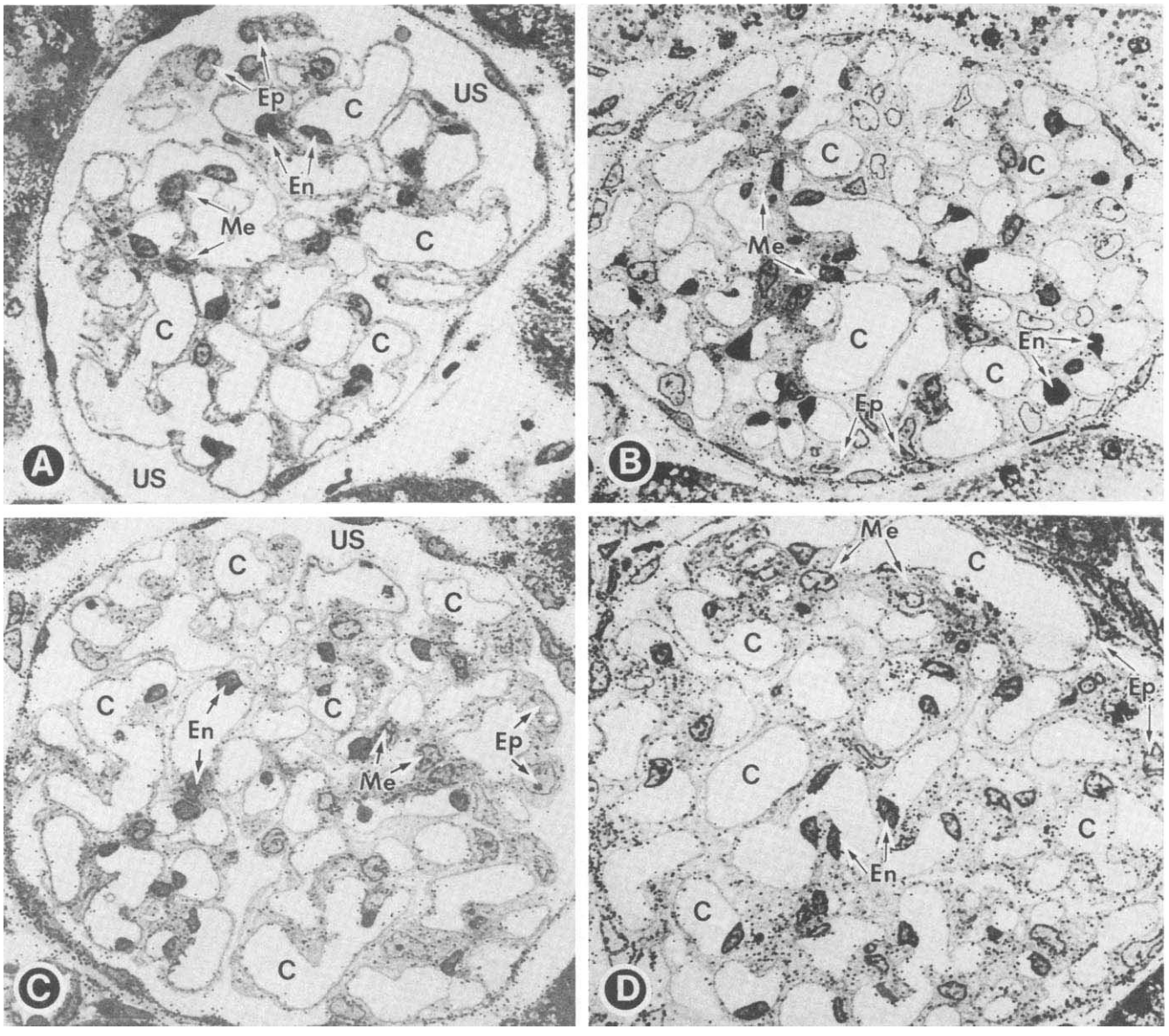

Fig. 6. *Light microscopic autoradiograms of [^{35}S]sulfate-labelled glomeruli from control* (**A**) *and IGF-1-treated kidneys* (**B–D**). A graded increase in the concentration of grains over the mesangium (Me) is seen with increasing concentrations of IGF-1 (panel B = 62.5 ng/ml, C = 125 ng/ml, and D = 625 ng/ml). Abbreviations are: Ep, epithelium; En, endothelium; C, capillary; US, urinary space. × 800.

Table 2. Tissue autoradiographic grain density of various glomerular compartments

	Epithelium	Endothelium	Mesangium[a]	GBM
Sulfate				
Control	0.66 ± 0.21	0.71 ± 0.34	0.74 ± 0.30	1.48 ± 0.41
IGF *125 ng/ml*	0.68 ± 0.19	0.85 ± 0.31	1.28 ± 0.26	2.25 ± 0.49[b]
IGF *375 ng/ml*	0.81 ± 0.20[b]	1.01 ± 0.25[b]	1.87 ± 0.29[c]	2.45 ± 0.38[b]
IGF *625 ng/ml*	0.88 ± 0.35[b]	1.27 ± 0.39[b]	2.29 ± 0.37[c]	2.81 ± 0.45[b]
Methionine				
Control	2.38 ± 0.49	1.94 ± 0.31	2.03 ± 0.41	4.10 ± 0.57
IGF *375 ng/ml*	2.68 ± 0.46	2.50 ± 0.51	3.29 ± 0.51[b]	4.83 ± 0.59

Thirty-five samples were used per variable for morphometric analyses.
[a] Mesangium includes extracellular matrix as well as the mesangial cell
[b] $P < 0.01$
[c] $P < 0.001$

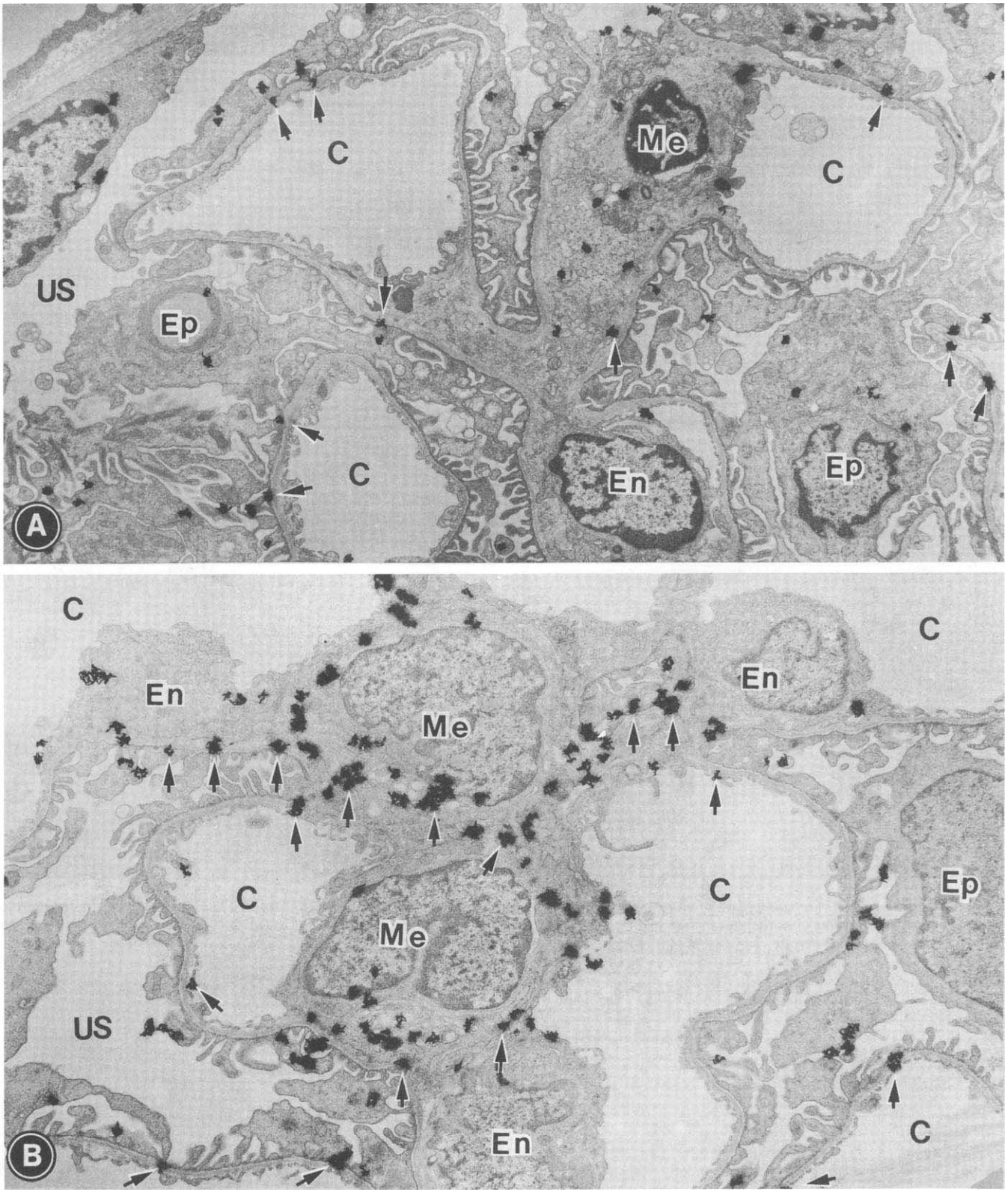

Fig. 7. *Electron microscopic autoradiograms of [^{35}S]sulfate-labelled glomeruli from control* (**A**) *and IGF-1-treated kidneys* (**B**). A notable increase in the concentration of autoradiographic grains (arrows) overlying the mesangium (Me) is observed with the treatment of IGF-1 (625 ng/ml). Abbreviations are: Ep, epithelium; En, endothelium; C, capillary; US, urinary space. × 9,000.

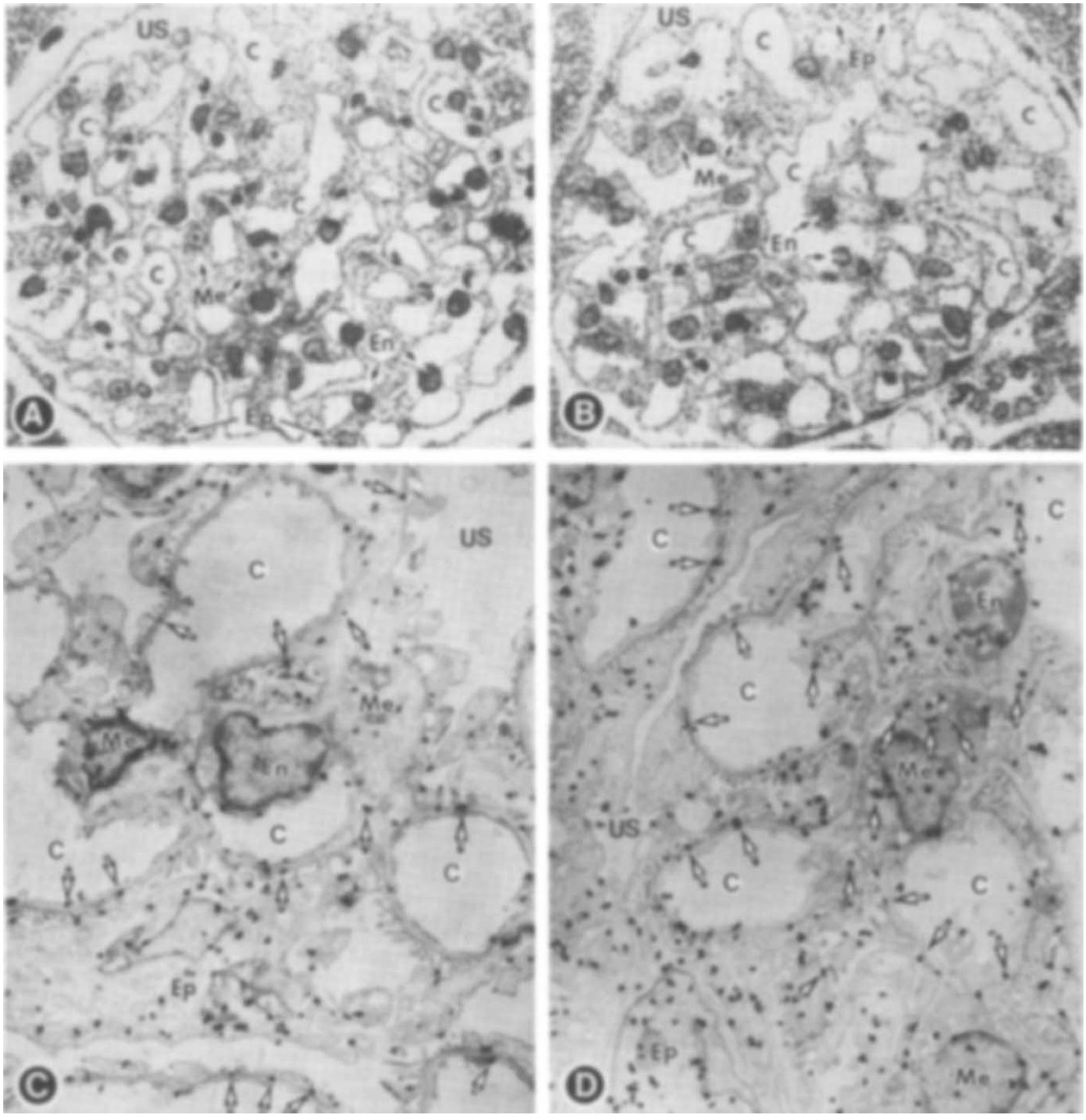

Fig. 8. *Light (***A and B***) and electron (***C and D***) microscopic autoradiograms of [^{35}S]methionine-labelled glomeruli from control (panels A and C) and IGF-1-treated (panels B and D) kidneys.* A generalized increase in the concentration of autoradiographic grains (arrows) is observed with the treatment of IGF-1 (375 ng/ml), and is more so over the mesangial (Me) regions. Abbreviations are: Ep, epithelium; En, endothelium; C, capillary; US, urinary space. A and B × 800; C and D × 3,000.

the three to fourfold increase in the autoradiographic grain density (Table 2) indicative of *de novo* sulfated glycoprotein synthesis suggests somewhat of a selective responsiveness of the mesangial cell in a *milieu* where "all" the cell types of the glomerulus are "equally" exposed to IGF-1. The effect of IGF-1 most likely occurs via receptor-mediated mechanisms. In this regard, the receptors for insulin and insulin like growth factors have been reported in isolated glomeruli [7, 44]. Furthermore, receptors for IGF-1 have been demonstrated on cultured mesangial cells [8–10], whereas their presence on the other cell types of glomerulus is unknown.

Via receptor-mediated mechanisms, the mesangial cells respond to various hormones or growth factors, for example, TGF-β and IGF. TGF-β has been shown to modulate the

Table 3. [^{35}S]-methionine associated radioactivity in tissue and media fractions[a]

	Control	IGF-1-treated[b]
Media ($N = 5$)	$2.41 \pm 0.11 \times 10^6$ dpm/ml	$2.75 \pm 0.12 \times 10^6$ dpm/ml[b]
Glomerular ($N = 5$)	$4.37 \pm 0.23 \times 10^8$ dpm/kidney	$5.01 \pm 0.30 \times 10^8$ dpm/kidney[b]

[a] The IGF concentration in the perfusion medium was 375 ng/ml
[b] $P < 0.05$

Table 4. Relative percentage increase of [^{35}S]-methionine and [^{35}S]-sulfate associated incorporated radioactivity with exposure to the same concentration of IGF

Fractions	[^{35}S]-methionine	[^{35}S]-sulfate
Glomerular ($N = 5$)	15.4 ± 1.9	65.1 ± 4.6
Media ($N = 5$)	14.3 ± 1.1	126.9 ± 7.3

The IGF concentration used in the perfusion medium was 375 ng/ml

functions of mesangial cells and thus play an important role in the proliferative form of experimental glomerulonephritis and glomerulosclerosis [42, 43]. Along these lines, it is conceivable that the biosynthetic functions of the mesangial cells can be modulated by the high concentration of IGF-1 in the serum in diabetes via receptor-mediated mechanism [45]. By this mechanism, sustained high serum levels of IGF-1 in vivo could result in glomerular hypertrophy and a generalized increase in the synthesis of various extracellular matrix proteins in the mesangium. Such a generalized effect on protein synthesis was noted in our present ex vivo studies, where some increase in the incorporation of [^{35}S]-methionine was observed following a short exposure (9 hr) to IGF-1. With comparable doses of IGF-1, PG synthesis determined by [^{35}S]-sulfate labeling was proportionately greater than total protein synthesis ascertained by [^{35}S]-methionine labeling (Table 4). This suggests that IGF-1 had a greater or more selective effect in stimulating the PG's biosynthesis.

With respect to diabetes, several investigators have reported a decreased *de novo* synthesis of proteoglycans in late stage nephropathy [21–24]. In the late stage of diabetes, there is an excessive accumulation of extracellular matrix and consequential mechanical compression of mesangial cells, which expectedly would interfere with their biosynthetic functions. This may account for decreased *de novo* synthesis of PGs. Also, there is growing evidence that sustained hyperglycemia itself inhibits the biosynthesis of PGs [46]. Finally, the decreased *de novo* biosynthesis of PGs has been investigated mostly in the streptozotocin-induced diabetic model in which low, rather than high, levels of IGF in the serum were detected [47, 48]. Thus, the decreased biosynthesis of PGs in later stages of diabetes may be multifactorial in origin.

Altered biosynthesis of any constitutive matrix glycoprotein would disrupt its interactions with other matrix components, and result in defective assemblage and organization of extracellular matrix [49, 50]. An abnormal matrix may also form if defective macromolecules are not retained on the cell surface in order to mediate proper cell-matrix interactions but are released into the extracellular compartment [49, 50]. In this study IGF-1 also induced the following abnormalities in the synthesis of proteoglycans: an increased number of GAG-chains were released into the media fraction, and PGs/GAGs in the media exhibited a lower charge-density (0.35 to 0.55 vs. 0.4 to 0.6 M, Fig. 5). The increased number of GAG chains in the media could be due to augmented synthesis and/or enhanced turnover (synthesis/degradation) of the proteoglycans under the influence of IGF-1. Persistence of these biochemical abnormalities over a "prolonged period" could also contribute to defective assemblage and organization of various matrix glycoproteins, resulting in the formation of abnormal extracellular matrices. Also, biosynthetic abnormalities in the proteoglycans, especially those present on the cell surfaces, may perturb the cell-matrix interactions which ultimately would lead to a compromise in the filtration functions of the capillaries during the later stages of diabetes [40].

In summary, IGF-1 induces alterations in the *de novo* synthesis of proteoglycans, in particular that of the chondroitin/dermatan sulfate, by differentially affecting the biosynthetic functions of the mesangial cell of the renal glomerulus. These IGF-1 induced biochemical changes may have a role in the pathogenesis of the initial stages of diabetic nephropathy.

Acknowledgments

This research was supported by NIH Grant DK 28492. We thank Dr. Frank A. Carone for critically editing the manuscript.

Reprint requests to Yashpal S. Kanwar, M.D., Ph.D., Department of Pathology, Northwestern University Medical School, 303 E. Chicago Avenue, Chicago, Illinois 60611, USA.

References

1. Baxter RC: The somatomedins: Insulin-like growth factors. *Adv Clin Chem* 25:49–115, 1986
2. Clemmons DR, Underwood LE, Van Wyk JJ: Hormonal control of immunoreactive somatomedin production by cultured human fibroblasts. *J Clin Invest* 67:10–19, 1981
3. Zapf J, Froesch ER, Humbel RE: The insulin-like growth factor of human serum: Chemical and biological characterization and aspects of their possible physiological role. *Curr Top Cell Regul* 19:257–309, 1981
4. Abrass CK, Raugi LS, Gabourel LS, Lovett DH: Insulin and insulin-like growth factor-1 binding to cultured rat glomerular mesangial cells. *Endocrinology* 123:2432–2439, 1988
5. Canalis E: Effect of insulin-like growth factor-1 on DNA and protein synthesis in cultured rat calvaria. *J Clin Invest* 66:709–719, 1980
6. Froesch ER, Zapf J: Insulin-like growth factors and insulin: Comparative aspects. *Diabetologia* 28:485–493, 1985
7. Pillion DJ, Haskell JF, Meezan E: Distinct receptors for insulin-like growth factor-1 in rat renal glomeruli and tubules. *Am J Physiol* 254:E504–E512, 1988
8. Conti FG, Striker LJ, Lesniak MA, MacKay K, Roth J, Striker GE: Studies on binding and mitogenic effect of insulin and insulin-like growth factor-1 in glomerular mesangial cells. *Endocrinology* 122:2788–2795, 1988
9. Conti FG, Striker LJ, Elliot SJ, Andreani D, Striker GE: Synthesis and release of insulin-like growth factor-1 by mesangial cells in culture. *Am J Physiol* 254:F1214–F1219, 1988
10. Doi T, Striker LJ, Elliot SJ, Conti FG, Striker GE: Insulin-like growth factor-1 is a progression factor for human mesangial cells. *Am J Pathol* 134:395–404, 1989

11. STILES AD, SOSENKO RS, D'ERCOLE AJ, SMITH BT: Relation of kidney tissue somatomedin-C/insulin-like growth factor-1 to post-nephrectomy renal growth in rat. *Endocrinology* 117:2397–2401, 1985
12. FLYBJERG A, USSING T, NAERAA R, INGERSELV J, ORSKOV H: Kidney tissue somatomedin C and initial renal growth in diabetic and uninephrectomized rats. *Diabetologia* 31:310–314, 1988
13. ANDERSON GL, SKOTTNER A, JENNISCHE E: Immunocytochemical and biochemical localization of insulin-like growth factor-1 in the kidney of rats before and after uninephrectomy. *Acta Endocrinol* (Copenh) 119:555–560, 1988
14. KANWAR YS, FARQUHAR MG: Presence of heparan sulfate in glomerular basement membrane. *Proc Natl Acad Sci USA* 76:1301–1307, 1979
15. KANWAR YS, HASCALL VC, FARQUHAR MG: Partial characterization of newly synthesized proteoglycans isolated from the glomerular basement membrane. *J Cell Biol* 90:527–532, 1981
16. MARTIN GR: Laminin and other basement membrane components. *Ann Rev Cell Biol* 3:57–85, 1987
17. BRUIJN JA, HOGENDOORN PCW, HOEDEMAEKER PJ, FLEUREN GJ: The extracellular matrix in pathology. *J Lab Clin Med* 111:140–149, 1988
18. CAGLIERO E, MAIELLO M, BOERI D, ROY S, LORENZI M: Increased expression of basement membrane component in human endothelial cells cultured in high glucose. *J Clin Invest* 82:735–738, 1988
19. AYO SH, RADNIK RA, GARONI JA, GLASS WF, KREISBERG JI: High glucose causes an increase in extracellular matrix proteins in cultured mesangial cells. *Am J Pathol* 136:1339–1348, 1990
20. POULSOM R, KURKINEN M, PROCKOP DJ, HANDFORD RPB: Increased steady-state levels of laminin B1 mRNA in kidneys of long-term streptozotocin-diabetic rats. *J Biol Chem* 263:10072–10076, 1988
21. KANWAR YS, ROSENZWEIG LJ, LINKER A, JAKUBOWSKI M: Decreased de novo synthesis of glomerular proteoglycans in diabetes: Biochemical and autoradiographic evidence. *Proc Natl Acad Sci USA* 80:2272–2275, 1983
22. PARTHASARATHY N, SPIRO RG: Effect of diabetes on the glycosaminoglycan component of the glomerular basement membrane. *Diabetes* 31:738–741, 1982
23. KLEIN DJ, BROWN DM, OEGAMA TR: Glomerular proteoglycans in diabetes. Partial structural characterization and metabolism of de novo synthesized heparan $^{35}SO_4$ and dermatan-$^{35}SO_4$ proteoglycans in streptozotocin induced diabetic rats. *Diabetes* 35:1130–1142, 1986
24. ROHRBACH DH, WAGNER CW, STAR VL, MARTIN GR, BROWN KS: Reduced synthesis of basement heparan sulfate proteoglycans in streptozotocin-induced diabetic mice. *J Biol Chem* 258:11672–11677, 1983
25. DECKERT T, RASMUSSEN BF, DJURUP R, DECKERT M: Glomerular size and charge selectivity in insulin-dependent diabetes mellitus. *Kidney Int* 33:100–106, 1988
26. GRANT M, RUSSELL B, FITZGERALD C, MERIMEE TJ: Insulin-like growth factors in vitreous. Studies in control and diabetic subjects with neovasculization. *Diabetes* 35:416–420, 1986
27. MAUER SM, STEFFES MW, ELLIS EN, SUTHERLAND DER, BROWN DM, GOETZ FC: Structural-functional relationships in diabetic nephropathy. *J Clin Invest* 74:1143–1155, 1984
28. SCHALKWIJK J, JOOSTEN LAB, BERG WB, WYK JJV, PUTTE LBA: Insulin-like growth factor stimulation of chondrocyte proteoglycan synthesis by human synovial fluid. *Arthr Rheum* 32:66–71, 1989
29. LUYTEN FP, HASCALL VC, NISSLEY P, MORALES TI, REDDI AH: Insulin-like growth factors maintain steady-state metabolism of proteoglycans in bovine articular cartilage explants. *Arch Biochem Biophys* 267:416–425, 1988
30. KANWAR YS, ROZENZWEIG LJ, JAKUBOWSKI MJ: Distribution of de novo synthesized sulfate glycosaminoglycans in the glomerular basement membrane and mesangial matrix. *Lab Invest* 50:216–225, 1983
31. KANWAR YS, HASCALL VC, JAKUBOWSKI ML, GIBBONS JT: Effect of beta-D-xyloside on glomerular proteoglycans. 1. Biochemical studies. *J Cell Biol* 99:715–722, 1984
32. HOLTHOFER H, SAINIO K, MIETTINEN A: Rat glomerular cells do not express podocytic markers when cultured in vitro. *Lab Invest* 65:548–557, 1991
33. KANWAR YS, FARQUHAR MG: Isolation of glycosaminoglycans (heparan sulfate) from glomerular basement membranes. *Proc Natl Acad Sci USA* 76:4493–4497, 1979
34. HEINEGARD D, HASCALL VC: Characterization of chondroitin sulfate isolated from trypsin-chymotrypsin digests of cartilage proteoglycans. *Arch Biochem Biophys* 16:427–441, 1974
35. WASTESON A: A method for determination of molecular weight and molecular weight distribution of chondroitin sulfate. *J Chromatogr* 59:87–97, 1971
36. ROSENZWEIG LJ, KANWAR YS: Removal of sulfated (heparan sulfate) or non-sulfated (hyaluronic acid) glycosaminoglycans results in increased permiability of glomerular basement membrane to ^{125}I-bovine serum albumin. *Lab Invest* 47:177–184, 1980
37. SALPETER MM, BACHMAN L: Autoradiography, in *Principles and Techniques in Electron Microscopy: Biological Applications*, edited by MA HAYAT, New York, Van Nostrand Reinhold, Volz, 1972, pp. 221–278
38. WEIBEL ER: The value of stereology in analyzing structure and function of cells and organs. *J Microscop* 95:3–13, 1972
39. ARON DC, ROSENZWEIG JL, ABBOUD HE: Synthesis and binding of insulin-like growth factor-1 by human glomerular mesangial cells. *J Clin Endocrinol Metab* 68:585–591, 1989
40. KANWAR YS, LIU ZZ, KASHIHARA N, WALLNER EI: Current status of the structural and functional basis of glomerular filtration and proteinuria. *Semin Nephrol* 11:390–413, 1991
41. MCCARTHY KJ, ACCAVITTI MA, COUCHMAN JR: Immunological characterization of a basement membrane-specific chondroitin sulfate proteoglycan. *J Cell Biol* 109:3187–3198, 1989
42. BORDER WA, OKUDA S, LANGUINO LR, RUOSLAHTI E: Transforming growth factor regulates production of proteoglycans by mesangial cells. *Kidney Int* 37:689–695, 1990
43. OKUDA S, LANGUINO LR, RUOSLAHTI E, BORDER WA: Elevated expression of transforming growth factor and proteoglycan production in experimental glomerulonephritis. *J Clin Invest* 86:453–462, 1990
44. CONTI FG, ELLIOT SJ, STRIKER LJ, STRIKER GE: Binding of insulin-like growth factor-1 by glomerular endothelial and epithelial cells: Further evidence for IGF-1 action in the renal glomerulus. *Biochem Biophys Res Commun* 163:952–958, 1989
45. COHEN MP, JASTI K, RYE DL: Somatomedin in insulin dependent diabetes mellitus. *J Clin Endocrinol Metab* 45:236–239, 1977
46. LIU Z, YOSHINAGA Y, KANWAR YS: Influence of sugars on metanephric development. *J Am Soc Nephrol* 2:578, 1991
47. MAES M, KETELSLEBERG JM, UNDERWOOD LE: Low plasma somatomedin-C in streptozotocin-induced diabetes mellitus. *Diabetes* 32:1060–1069, 1983
48. MAES M, UNDERWOOD LE, KETELSLEBERG JM: Low serum somatomedin-C in insulin dependent diabetes: Evidence for a post-receptor mechanism. *Endocrinology* 118:377–382, 1986
49. YURCHENCO PD, FURTHMAYR H: Self-assembly of basement membrane collagen. *Biochemistry* 23:1839–1850, 1984
50. YURCHENCO PD, TSILIBARY EC, CHARONIS AS, FURTHMAYR H: Models for the self-assembly of basement membrane. *J Histochem Cytochem* 34:93–102, 1986